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SCORE2-DIABETES AND CARDIOVASCULAR RISK: INSIGHTS FROM TRADITIONAL AND NON-TRADITIONAL BIOMARKERS

SCORE2-DIJABETES I KARDIOVASKULARNI RIZIK: TRADICIONALNI I NOVI BIOMARKERI

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Summary

Background: Cardiovascular (CV) disease is the major cause of death in type 2 diabetes mellitus (T2DM). The SCORE2-diabetes is a recently developed algorithm that improves CV risk assessment in patients with T2DM. The study aimed to explore the relationship between SCORE2-Diabetes and clinical and biochemical parameters in individuals with T2DM.
Methods: A total of 106 T2DM patients were evaluated in this cross-sectional study. SCORE2-Diabetes (%) was calculated, and associations with clinical and biochemical parameters were analysed using correlation and logistic regression analysis. Principal component analysis (PCA) was applied to group different cardiometabolic risk biomarkers into several factors.
Results: In multivariate logistic regression analysis, retinol-binding protein 4 (RBP4) (OR 1.08, 95% CI 1.03–1.13, p=0.001), urinary albumin excretion (UAE) (OR 1.01, 95% CI 1.00–1.01, p=0.023), and triglycerides (TG) (OR 2.91, 95% CI 1.12–7.57, p=0.028) were inde-

Kratak sadržaj

Uvod: Kardiovaskularne (KV) bolesti su glavni uzrok smrti kod pacijenata sa dijabetes melitusom tip 2 (T2DM). SCORE2-Dijabetes je nedavno razvijen algoritam koji poboljšava procenu KV rizika kod pacijenata sa T2DM. Cilj studije bio je da se ispita povezanost SCORE2-Dijabetesa i kliničkih i biohemijskih parametara kod osoba sa T2DM.
Metode: Ukupno 106 pacijenata sa T2DM je uključeno u studiju preseka. Izračunat je SCORE2-Dijabetes (%), a povezanost sa kliničkim i biohemijskim parametrima je analizirana korišćenjem korelacione i logističke regresione analize. Analiza glavnih komponenti (PCA) je primenjena da bi se različiti biomarkeri kardiometaboličkog rizika grupisali u nekoliko faktora.
Rezultati: U multivarijantnoj logističkog regresionoj analizi, retinol-vezujući protein 4 (RBP4) (OR 1,08, 95% CI 1,03–1,13, p=0,001), albumin u urinu (UAE) (OR 1,01, 95% CI 1,00–1,01, p=0,023) i triglyceridi (TG) (OR 2,91, 95% CI 1,12–7,57, p=0,028) su nezavisno povezani sa SCORE2-Dijabetes-om. ROC analiza je pokazala visoku

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pendently correlated with SCORE2-Diabetes. ROC analysis showed high diagnostic accuracy for UAE (AUC=0.894) and RBP4 (AUC=0.871), while TG showed moderate accuracy (AUC=0.713) for identifying patients with high cardiovascular risk according to the SCORE2-Diabetes. Univariate binary logistic regression analysis showed that 2 PCA factors are significantly associated with the high SCORE 2-Diabetes value (above 45%). Cardiovascular-kidney-metabolic factors (i.e., RBP4, TG, Cystatin C, and UAE) had higher discriminative capability for high SCORE 2-Diabetes value (above 45%) than the inflammation factor (i.e., hsCRP) (p<0.001 vs. p=0.008, respectively).

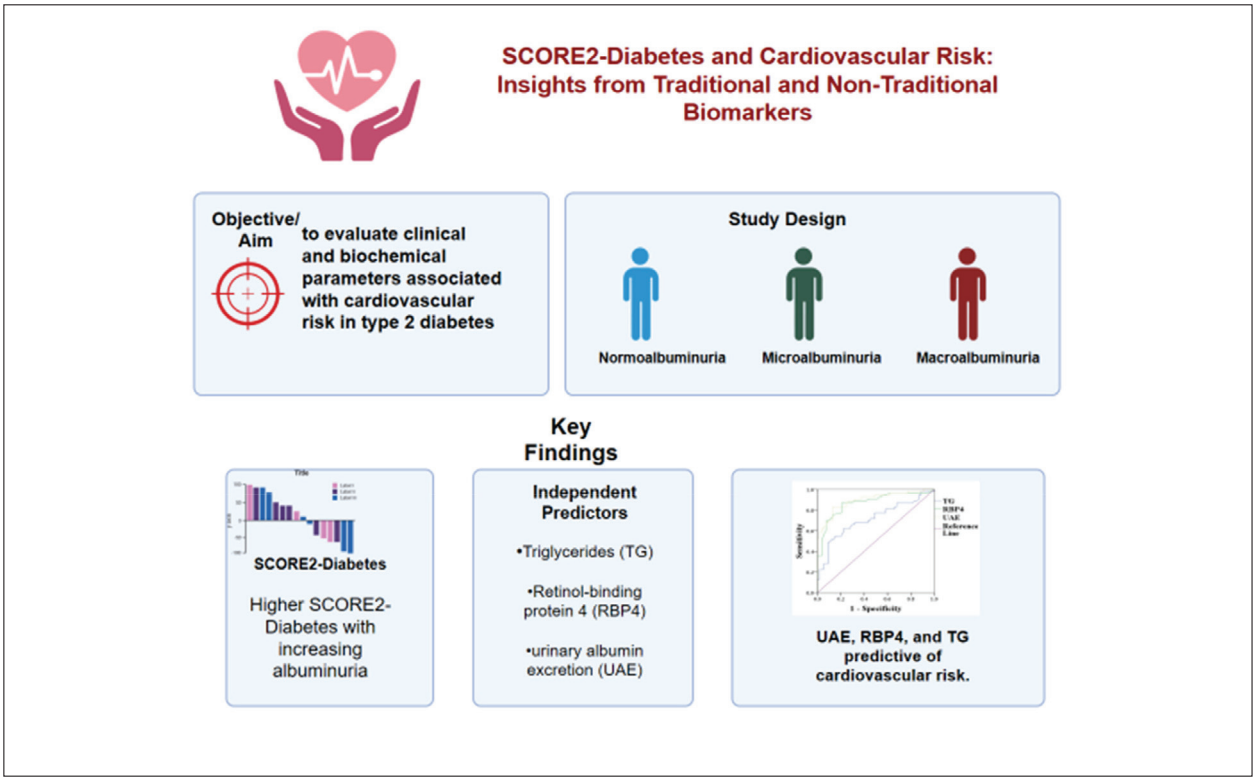
Conclusion: Elevated RBP4 and UAE are independently associated with an increased SCORE2-Diabetes risk. Incorporation of these biomarkers might refine cardiovascular risk stratification in T2DM and enable early intervention. Prospective studies are needed to confirm such results and assess therapeutic implications.

Keywords: type 2 diabetes mellitus, SCORE2-Diabetes, cardiovascular risk, biomarkers

dijagnostičku tačnost za UAE (AUC=0,894) i RBP4 (AUC=0,871), dok je TG pokazao umerenu tačnost (AUC=0,713) za identifikaciju pacijenata sa visokim kardiovaskularnim rizikom prema SCORE2-Dijabetesu. Univarijantna binarna logistička regresiona analiza pokazala je da su 2 PCA faktora značajno povezana sa visokom vrednošću SCORE 2-Dijabetesa (iznad 45%). Kardiovaskularno-bubrežno-metabolički faktor (RBP4, TG, cistatin C i UAE) imao je veću diskriminativnu sposobnost visokih vrednosti SCORE 2-Dijabetesa (iznad 45%) od faktora inflamacije (hsCRP) (p<0,001 naspram p=0,008, respektivno).

Zaključak: Povišeni RBP4 i UAE su nezavisno povezani sa SCORE2-Dijabetesom. Uključivanje ovih biomarkera moglo bi da poboljša stratifikaciju kardiovaskularnog rizika kod dijabetesa tip 2 i omogući ranu intervenciju. Potrebne su prospektivne studije kako bi se potvrdili takvi rezultati i procenili terapijski efekti.

Ključne reči: dijabetes melitus tip 2, SCORE2-Dijabetes, kardiovaskularni rizik, biomarkeri



Introduction

Type 2 diabetes mellitus (T2DM) continues to be a critical public health concern worldwide due to its increasing prevalence and associated complications (1). Microvascular and macrovascular complications resulting from chronic hyperglycaemia are significant contributors to the morbidity and mortality of the disease (2). In particular, cardiovascular diseases (CVD) are the major cause of death among individuals with diabetes (3). Therefore, accurately predicting cardiovascular risk at the earliest phase is important for developing appropriate treatment and prevention strategies (4).

In 2023, the SCORE2-Diabetes model was introduced to address this need by incorporating diabetes-specific parameters – such as age at di-

agnosis, glycated haemoglobin (HbA1c), and renal function assessed by estimated glomerular filtration rate (eGFR) into the original SCORE system, which was used to evaluate cardiovascular risk in the general population (5). This model enables classification of individuals with T2DM based on their 10-year risk of major adverse cardiovascular events (MACE) (5). SCORE2-Diabetes is particularly intended for individuals with no symptoms of atherosclerotic cardiovascular disease (ASCVD) or without severe target organ damage (5). However, to provide a more accurate and personalized risk prediction, it is still necessary to complement this algorithm with additional biochemical and clinical markers (6, 7). In this context, emerging parameters such as retinol-binding protein 4 (RBP4), cystatin C, and triglyceride-glucose index (TyG) have gained attention as potential cardiometabolic risk indicators in individuals with T2DM (7–20).

RBP4 is a transport protein primarily secreted by the liver and adipocytes, and it plays a major role in the transport of retinol (vitamin A) (21). In recent years, however, it has garnered increasing attention as a biomarker in cardiometabolic disorders due to its close association with insulin resistance (22). Elevated levels of RBP4 have been linked to conditions such as T2DM, obesity, and metabolic syndrome, and it has been suggested that RBP4 may play a role in atherogenesis (23–25). Some studies have reported a correlation between high RBP4 concentrations and both endothelial dysfunction and systemic inflammation (8).

Cystatin C is a low-molecular-weight cysteine protease inhibitor and is used as an alternative biomarker for estimating GFR (26). It has recently been shown that cystatin C is not only a biomarker of renal function but may also predict cardiovascular events. In individuals with diabetes, elevated cystatin C levels were correlated with an increased risk of cardiovascular mortality (26, 27). This suggests that cystatin C may play a dual role in both renal function assessment and cardiovascular risk prediction.

The TyG index is a parameter derived from the logarithmic combination of fasting triglycerides (TG) and fasting glucose levels, and is commonly considered an indirect marker of insulin resistance (11). Several studies have reported that the TyG index is associated with increased cardiovascular risk in individuals with both metabolic syndrome and T2DM (14–17, 20, 28, 29). Some investigations have suggested that the TyG index has predictive power comparable to, or even superior to, that of the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) (28). As a result, the TyG index and its combination with obesity indices, such as body mass index (BMI) and waist circumference (WC) (e.g., TyG-BMI and TyG-WC), are increasingly used as practical markers of cardiovascular risk (18, 28, 30).

Additionally, traditional markers of renal injury, such as urinary albumin excretion (UAE), are not only indicative of nephropathy. Still, they are also recognized as predictors of systemic vascular damage and, consequently, cardiovascular events. Indeed, the presence of micro- and macroalbuminuria has been recognized as a strong risk factor that adversely affects prognosis in individuals with diabetes (31).

This study aims to comprehensively explore the clinical and biochemical parameters associated with the SCORE2-Diabetes score in individuals with T2DM. Moreover, by classifying patients by UAE level, we aimed to assess the impact of albuminuria on cardiovascular risk.

Materials and Methods

Participants

A total of 106 individuals with T2DM were consecutively recruited in this cross-sectional study during their regular check-ups at the Primary Health Care Centre in Podgorica, Montenegro. The study extends our previous work examining cardiometabolic risk factors in diabetes (31). The endocrinologist conducted patient recruitment during their health checkup. The research was approved by the Institutional Ethical Committee (number 317/2–3). The investigation was conducted in accordance with the Declaration of Helsinki, and each participant provided written informed consent.

Participants were eligible for inclusion if they had T2DM, were aged 40–69 years, and had no urinary infection, acute inflammatory disease, or haematuria. The exclusion criteria were as follows: type 1 diabetes mellitus; a history of percutaneous coronary intervention, myocardial infarction, unstable angina, ischemic heart disease, or atrial fibrillation; hsCRP>10 mg/L; other conditions associated with proteinuria (e.g., amyloidosis or vasculitis); eGFR<15 mL/min/1.73 m^2; kidney transplantation; chronic dialysis; renal disease other than diabetic nephropathy; stroke; acute myocardial infarction; malignant diseases; pregnancy; and ethanol consumption >20 g/day.

Biochemical analyses

Venipuncture was performed on each participant after an overnight fast. Biochemical analyses [i.e., fasting glucose, creatinine, HbA1c, total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c)] were determined on an automatic analyser (Roche Cobas 400, Mannheim, Germany). The UAE was measured by the immunoturbidimetric method on the same analyser.

High-sensitivity C-reactive protein (hsCRP), cystatin C, and RBP4 levels were measured by nephelometry using a Behring Nephelometer Analyser (Marburg, Germany).

Patients were instructed on how to collect a 24-hour urine sample. A UAE level of <30 mg/24 h was defined as normoalbuminuria, levels between 30 and 300 mg/24 h as microalbuminuria, and levels ≥300 mg/24 h as macroalbuminuria. Blood pressure, body weight, and body height were measured, whereas body mass index (BMI) was calculated.

The TyG index (20), TyG-BMI (30), and atherogenic index of plasma (AIP) (32) were calculated as follows:

AIP = log [TG (mmol/L)/HDL-C (mmol/L)];

TyG = log [TG (mg/dL) x glukoza (mg/dL)]/2;

TyG-BMI = TyG x BMI.

Glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (CKD-EPI 2021 creatinine equation) (33).

Systematic Coronary Risk Evaluation 2-Diabetes (SCORE2-Diabetes, %) predicts 10-year CVD risk in individuals with T2DM (https://www.mdcalc.com/calc/10510/score2-diabetes) (5). This score includes the following risk factors: age, systolic blood pressure (SBP), smoking, TC, and HDL-c, and three T2DM-specific variables: HbA1c, age at diabetes onset, and estimated glomerular filtration rate ($eGFR_{CKD\text{-}EPI\ 2021\ creatinine\ equation}$).

Statistical analysis

The statistical analysis was performed using SPSS statistical software (IBM SPSS Statistics, version 24). The Shapiro-Wilk test was used to examine the distribution of the continuous variables. Data are presented as mean ± standard deviation for variables that follow normal distribution and median (interquartile range) for those that do not. Categorical variables were presented as absolute frequencies. Comparisons among groups defined by the UAE rate were performed using the Kruskal-Wallis test. Associations between continuous variables were assessed using Spearman's correlation analysis. Univariate logistic regression was used to examine the predictive power of the overall estimated parameters for detecting cardiovascular risk. Multivariate logistic regression analysis was used to identify independent predictors. Data were presented as odds ratio (OR) and 95% confidence interval (CI) (lower limit/upper limit). The Nagelkerke R^2 value was used to explain the variation in the dependent variable. Diagnostic characteristics of the independent correlations were examined using receiver operating characteristic (ROC) analysis. The area under the curve (AUC) indicates the probability that an event will be correctly predicted. The optimal test cutoff value was determined using the classification table, based on the sensitivity closest to 1 and 1 − specificity closest to 0. Factorial analysis was performed using principal component analysis (PCA) with varimax rotation and Kaiser normalization to group cardiometabolic risk biomarkers into several factors. A P-value < 0.05 was considered statistically significant.

Results

This research included 106 individuals with T2DM, categorized into normoalbuminuria (n=58), microalbuminuria (n=18), and macroalbuminuria (n=30) groups. As shown in *Table I*, the prevalence of smoking was significantly higher in patients with micro- and macroalbuminuria compared to those with normoalbuminuria (p<0.01). Diabetes duration was also significantly longer in the micro- and macroalbuminuria groups (p<0.001).

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were progressively higher across albuminuria categories (p<0.05), while BMI was significantly lower in the macroalbuminuria group compared to microalbuminuria (p=0.004). Patients with micro- and macroalbuminuria had higher glucose and HbA1c levels than those in the normoalbuminuria group (p<0.05).

Regarding lipid parameters, TG levels increased significantly with worsening albuminuria (p=0.004), whereas LDL-c, HDL-c, and TC showed no significant differences. Among the novel biomarkers, serum RBP4 and cystatin C levels were markedly elevated in the macroalbuminuria group compared with both normoalbuminuria and microalbuminuria (p<0.05 for all). Similarly, UAE and serum creatinine levels were highest, and eGFR was lowest, in patients with macroalbuminuria (all p<0.001).

The inflammatory marker hsCRP showed no significant differences among groups. TyG and AIP indices were significantly higher in micro- and macroalbuminuria compared to normoalbuminuria (p<0.05). Most importantly, the SCORE2-Diabetes (%) risk score increased progressively and significantly from normoalbuminuria to macroalbuminuria (p<0.001), indicating higher cardiovascular risk with increasing albuminuria.

Of all the examined parameters, divided by categories of albuminuria, only the values of SCORE2-Diabetes (%) showed a statistically significant difference between all three groups.

Table I Demographic characteristics and biochemical parameters of the analysed patients according to urinary albumin excretion rate category.

Parameter	Normoalbuminuria (N=58)	Microalbuminuria (N=18)	Macroalbuminuria (N=30)	P
Male/female (%)	38/62	23/67	43/57	0.779
Age (years)	64.45.5	65.36.3	65.98.6	0.283
T2DM duration (years)	3 (2–6)	7 (4–11)	7 (6–11)	0.007[a], <0.001[b], 0.940[c]
Smoker, No/Yes (%)	97/3	67/33	70/30	0.003[a], 0.001[b], 0.762[c]
BMI (kg/m^2)	27.62.6	27.32.2	26.01.95	0.680[a], 0.004[b], 0.074[c]
SBP (mmHg)	13211.3	14010	14914	0.007[a], <0.001[b], 0.011[c]
DBP (mmHg)	80 (77–86)	80 (75–83)	95 (84–101)	0.227[a], <0.001[b] 0.020[c]
Antihyperlipidemics, 1-No, 2-Yes (%)	62/38	44/56	70/30	0.212
Antihypertensives, 1-No, 2-Yes (%)	14/86	33/67	30/70	0.052
Oral antihyperglicemics-1, insulin-2, both 3 (%)	91/0/9	61/17/22	23/60/17	0.018[a], <0.001[b], 0.031[c]
Glucose (mmol/L)	7.4 (6.7–8.5)	10.7 (6.7–12.5)	9.1 (6.2–11.2)	0.031[a], 0.049[b], 0.412[c]
HbA1c (%)	7.2 (6.4–8.2)	8.5 (6.9–9.1)	8.4 (6.8–9.5)	0.081[a], 0.011[b], 0.999[c]
HbA1c (mmol/L)	55 (46–66.3)	69 (53–76)	68 (51–80)	0.081[a], 0.011[b], 0.999[c]
TC (mmol/L)	5.741.26	5.640.89	6.12 (5.16–8.14)	0.579
HDL-c (mmol/L)	1.320.38	1.26 0.30	1.200.34	0.503
LDL-c (mmol/L)	3.510.99	3.760.84	3.881.15	0.729
TG (mmol/L)	1.59 (1.31–2.04)	1.98 (1.21–2.51)	2.59 (1.35–3.24)	0.004[b]
TyG	1.80.4	2.20.6	2.31.0	0.280[a], 0.002[b], 0.233[c]
TyG-BMI	51.012.7	60.418.4	59.625.4	0.280[a], 0.025[b], 0.233[c]
AIP	0.10 (-0.06–0.24)	0.21 (-0.02–0.34)	0.36 (0.03–0.48)	0.007[a], <0.001[b], 0.940[c]
RBP4 (mg/L)	47 (40–58)	56 (41–70)	71 (64–92)	0.045[a], <0.001[b], 0.031[c]
Cystatin C (mg/L)	0.83 (0.75–0.99)	1.11 (0.84–1.29)	1.92 (1.64–2.33)	0.007[a], <0.001[b,c]
hsCRP (mg/L)	2.29 (1.05–3.61)	2.23 (0.57–6.07)	3.87 (1.37–6.41)	0.226
UAE (mg/24 h)	11.8 (7.77–17.67)	73.1 (49.6–90.7)	1278.6 (730.4–2704.5)	<0.001[a,b,c]
Creatinine (μmol/L)	65 (54–86)	86 (53–101)	162 (117–208)	0.031[a], <0.001[b,c]
eGFRCKD-EPI cre2021	99 (80–104)	74 (56–100)	32 (29–52)	0.105[a], <0.001[b,c]
eGFRCKD-EPI cre-CysC 2021	100 (78–109)	74 (57–99)	31 (27–45)	0.031[a], <0.001[b,c]
eGFRCKD-EPI CysC 2012	92 (72–101)	63 (52–90)	30 (24–38)	0.007[a], <0.001[b,c]
SCORE2-Diabetes (%)	27.8 (22.4–34.1)	40.4 (33.7–51.9)	57.6 (48.1–67.9)	<0.001[a,b,c]

[a]P<0.05-statistically significant difference between UAE-norm and microalbuminuria
[b]P<0.05-statistically significant difference between UAE-norm and macroalbuminuria
[c]P<0.05-statistically significant difference between microalbuminuria and macroalbuminuria
Abbreviations: UAE, urinary albuminuria excretion; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; TyG, triglycerides and glucose index; AIP, atherogenic Index of Plasma; RBP4, retinol binding protein 4; hsCRP, high-sensitivity C-reactive protein; eGFRCKD-EPI, Estimated Glomerular Filtration Rate Chronic Kidney Disease Epidemiology Collaboration; SCORE2-Diabetes, Systematic Coronary Risk Evaluation 2-Diabetes

Table II Spearman's correlation analysis between SCORE2-Diabetes and clinical parameters in individuals with type 2 diabetes.

Parameter	Rho (r)	P
Age (years)	0.537	<0.001
T2DM duration (years)	0.197	0.043
BMI (kg/m^2)	-0.224	0.021
SBP (mmHg)	0.339	<0.001
DBP (mmHg)	0.243	0.012
Glucose (mmol/L)	0.239	0.014
HbA1c (%)	0.330	0.001
HbA1c (mmol/L)	0.330	0.001
TC (mmol/L)	0.215	0.027
HDL-c (mmol/L)	-0.159	0.103
LDL-c (mmol/L)	0.227	0.019
TG (mmol/L)	0.436	<0.001
TyG	0.456	<0.001
TyG-BMI	0.405	<0.001
AIP	0.381	<0.001
RBP4 (mg/L)	0.633	<0.001
Cystatin C (mg/L)	0.762	<0.001
hsCRP (mg/L)	0.142	0.145
UAE (mg/24 h)	0.750	<0.001
Creatinine (mmol/L)	0.678	<0.001
eGFRCKD-EPI cre	-0.788	<0.001
eGFRCKD-EPI cre-CysC	-0.791	<0.001
eGFRCKD-EPI CysC	-0.797	<0.001

Abbreviations: UAE, urinary albuminuria excretion; T2DM, type 2 diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated haemoglobin; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; RBP4, retinol binding protein 4; hsCRP, high-sensitivity C-reactive protein; $eGFR_{CKD-EPI}$, estimated glomerular filtration rate Chronic Kidney Disease Epidemiology Collaboration; SCORE2-Diabetes, Systematic Coronary Risk Evaluation 2-Diabetes; TyG, triglycerides and glucose index; AIP, atherogenic index of plasma

Spearman's correlation analysis (*Table II*) revealed that SCORE2-Diabetes correlated positively with age ($p<0.001$), diabetes duration ($p=0.043$), SBP, DBP, glucose, HbA1c, TG, RBP4, cystatin C, UAE, creatinine, TyG, TyG-BMI, and AIP (all $p<0.05$). Conversely, BMI and eGFR (estimated using different equations) were negatively correlated with SCORE2-Diabetes (all $p<0.001$). The strongest positive correlations were observed for UAE ($\rho=0.750$), cystatin C ($\rho=0.762$), RBP4 ($\rho=0.633$), and serum creatinine ($\rho=0.678$), suggesting their close association with cardiovascular risk in T2DM patients.

Univariate logistic regression analysis (*Table III*) demonstrated that TG (OR 2.79, $p<0.001$), RBP4 (OR 1.11, $p<0.001$), and UAE (OR 1.01, $p=0.022$) were significantly associated with high cardiovascular risk, whereas BMI showed an inverse association with SCORE2-Diabetes (OR 0.83, $p=0.037$).

In multivariate logistic regression analysis, only TG (OR 2.91, $p=0.028$), RBP4 (OR 1.08, $p=0.001$), and UAE (OR 1.01, $p=0.023$) remained independently associated with cardiovascular risk, explaining 68.7% of the variation in SCORE2-Diabetes.

ROC curve analysis (*Figure 1, Table IV*) demonstrated excellent diagnostic accuracy for UAE (AUC=0.894) and RBP4 (AUC=0.871), while TG showed moderate accuracy (AUC=0.713) for identifying patients with high cardiovascular risk according to the SCORE2-Diabetes. Optimal cut-off values were 18.2 mg/24h for UAE (sensitivity 88.7%, specificity 73.6%), 50.5 mg/L for RBP4 (sensitivity 86.8%, specificity 71.7%), and 1.62 mmol/L for TG (sensitivity 69.8%, specificity 56.6%).

Overall, patients with higher albuminuria demonstrated significantly worse cardiometabolic profiles and higher SCORE2-Diabetes risk scores. Among all evaluated biomarkers, RBP4, UAE, and TG were independently associated with increased cardiovascular risk, with UAE and RBP4 demonstrating the strongest diagnostic performance.

Factorial analysis

The Kaiser-Meyer-Olkin index (KMO=0.644) and Bartlett's test of sphericity ($P<0.001$) confirmed analysis adequacy. The following parameters were included: RBP4 (mg/L), TG (mmol/L), cystatin C (mg/L), UAE (mg/24 h), and hsCRP (mg/L).

PCA extracted 2 factors, which explained 68% of the total variability, distributed as 40% and 28% across Factors 1 and 2, respectively. Results are presented in *Table V*. The first factor (cardiovascular-kidney-metabolic factor) included the following parameters: RBP4, TG, cystatin C, and UAE, except

Table III Logistic regression analysis for parameters associated with cardiovascular risk according to the SCORE2-Diabetes.

Univariate logistic regression analysis			
Predictors	Unadjusted OR	P	Nagelkerke R2
BMI (kg/m^2)	0.833 (0.702–0.989)	0.037	0.058
TG (mmol/L)	2.791 (1.569–4.964)	<0.001	0.197
RBP4 (mg/L)	1.112 (1.066–1.160)	<0.001	0.507
UAE (mg/24 h)	1.008 (1.001–1.016)	0.022	0.524
Multivariate logistic regression analysis			
BMI (kg/m^2)	0.826 (0.628–1.086)	0.170	0.687 (for Model)
TG (mmol/L)	2.910 (1.119–7.566)	0.028	
RBP4 (mg/L)	1.081 (1.033–1.131)	0.001	
UAE (mg/24 h)	1.007 (1.001–1.013)	0.023	

Abbreviations: BMI, body mass index; TG, triglycerides; RBP4, retinol binding protein 4; UAE, urinary albuminuria excretion.

Table IV Diagnostic characteristics of predictors of SCORE2-Diabetes.

Parameter	Area	Standard Error	Asymptotic Significance	95% CI	
				Lower Bound	Upper Bound
TG (mmol/L)	0.713	0.051	<0.0001	0.613	0.812
RBP4 (mg/L)	0.871	0.036	<0.0001	0.800	0.941
UAE (mg/24 h)	0.894	0.031	<0.0001	0.833	0.956

Abbreviations: TG, triglycerides; RBP4, retinol binding protein 4; UAE, urinary albuminuria excretion; CI- Confidence Interval

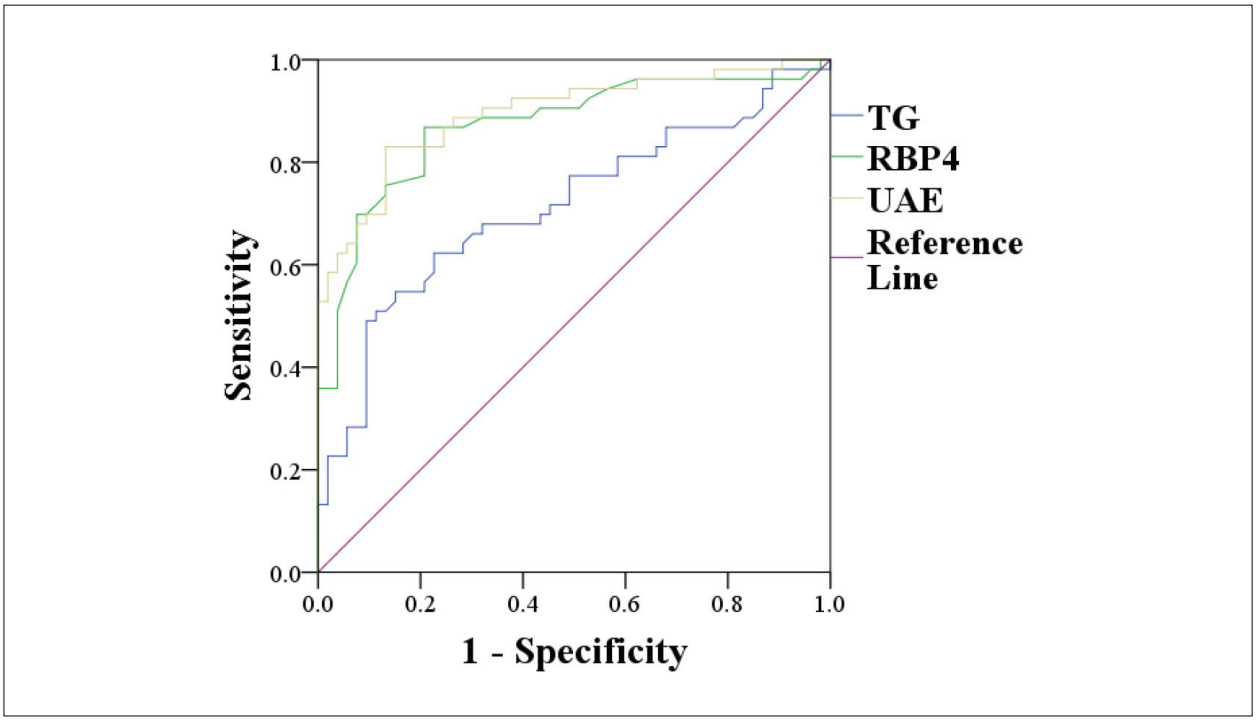


Figure 1 Graphical presentation of the ROC curve analysis and diagnostic characteristics of predictors of SCORE2-Diabetes.

Table V Factorial analysis in a cohort of patients with type 2 diabetes mellitus.

Factors	Factor's variables with loadings		Variability (%) Total: 68
Factor 1 (Cardiovascular-kidney-metabolic factor)	RBP4 (mg/L)	0.832	40
	TG (mmol/L)	0.743	
	cystatin C (mg/L)	0.641	
	UAE (mg/24 h)	0.575	
Factor 2 (Inflammation factor)	hsCRP (mg/L)	0.891	28

Abbreviations: TG, triglycerides; RBP4, retinol binding protein 4; UAE, urinary albuminuria excretion; hsCRP, high-sensitivity C-reactive protein

Table VI Binary logistic regression analysis of predictors – PCA extracted factors (scores) of SCORE2-Diabetes high value (>45%).

Predictors	B (SE)	Wald coefficient	OR (95% CI)	P
Factor 1 (Cardiovascular-kidney-metabolic factor)	4.057 (1.012)	16.065	57.8 (8.0–420.3)	<0.001
Factor 2 (Inflammation factor)	0.914 (0.347)	6.959	2.4 (1.3–4.9)	0.008

SE– standard error, OR– odds ratio (95th CI – confidence interval); P from binary logistic analysis

for hsCRP, which was included in the second factor (inflammation factor) alone.

Univariate binary logistic regression analysis showed that both PCA factors are significantly associated with the high SCORE 2-Diabetes value (above 45%). Precisely, Factor 1 (i.e., cardiovascular-kidney-metabolic factor that included RBP4, TG, cystatin C, and UAE) had higher discriminative capability than Factor 2 (i.e., inflammation factor that included hsCRP) according to their p-values (*Table VI*).

Discussion

In this study, we demonstrated that elevated serum RBP4, increased UAE, and higher TG levels were independently associated with cardiovascular risk as assessed by the SCORE2-Diabetes algorithm in patients with T2DM. ROC analysis showed high diagnostic accuracy for UAE (AUC=0.894) and RBP4 (AUC=0.871), while TG showed moderate accuracy (AUC=0.713) for identifying patients with high cardiovascular risk according to the SCORE2-Diabetes.

Previous research has shown that non-traditional biomarkers may enhance cardiovascular risk prediction in diabetic populations beyond conventional risk factors (8–10, 23, 24). Our results regarding RBP4 are consistent with earlier studies reporting its association with insulin resistance, systemic inflammation, and endothelial dysfunction – all of which contribute to atherosclerosis and adverse cardiovascular outcomes (9, 10, 23, 24). Under physiological conditions, vascular smooth muscle cells (VSMCs) express numerous contractile proteins necessary for vascular integrity and elasticity. A phenotypic modification of VSMCs is considered the first step preceding their proliferation and migration. These changes are related to lipid accumulation and enhanced production of extracellular matrix components, which further accelerate both the progression of atherogenesis and plaque instability (34). Elevated RBP4 has been shown to increase plaque instability by modulating VSMCs phenotype through the RhoA (Ras homolog family member A)/ROCK1 (Rho-associated coiled-coil-containing protein kinases) signalling (23).

Therefore, RBP4 may serve both as a biomarker and a potential therapeutic target in cardiometabolic disorders.

High UAE has been underused and underappreciated as a biomarker of CVD risk (35). Our results show that UAE, a well-established marker of renal microvascular injury (31, 35), strongly correlated with SCORE2-Diabetes risk. The SCORE2-Diabetes risk score showed an increasing trend from normoalbuminuria to macroalbuminuria, indicating higher cardiovascular risk with increasing albuminuria. Our results also show that even low-grade UAE (i.e., mi-

croalbuminuria) confers a significant cardiovascular risk (median SCORE2-Diabetes = 40.4%), underscoring its role in early risk detection.

Higher TG levels were independently associated with elevated cardiovascular risk, supporting the well-documented role of TG-rich lipoproteins in diabetic atherosclerosis (36, 37). Persistent hypertriglyceridemia promotes the formation of small dense LDL and increases atherogenicity, making TG an important target for risk reduction. Interestingly, while the TyG index and cystatin C demonstrated significant correlations with SCORE2-Diabetes, they did not remain independent predictors in multivariate analysis. This may be due to overlapping pathways with UAE and RBP4, or to an insufficient sample size to capture subtle effects. However, cystatin C and TG were grouped with RBP4 and UAE in PCA as cardiovascular-kidney-metabolic factors. This factor and the inflammation factor (which included hsCRP) are both significantly associated with the high SCORE 2-Diabetes value (above 45%). Moreover, Factor 1 (i.e., cardiovascular-kidney-metabolic factor) had higher discriminative capability than Factor 2 (i.e., inflammation factor). However, inflammation is a well-established underlying feature of diabetes (38, 39), suggesting that a multimarker approach could provide a deeper understanding of distinct metabolic pathways.

Based on our findings, the identification of RBP4 and UAE as independent predictors underscores their potential to improve SCORE2-Diabetes–based risk assessment. Incorporating these biomarkers into clinical algorithms might improve early detection of patients at high CVD risk and facilitate personalized interventions, including intensified lipid-lowering therapy, stricter glycaemic control, and the use of renoprotective agents such as glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose cotransporter-2 (SGLT2) inhibitors (2, 28). Furthermore, measuring RBP4 may provide insights into residual cardiovascular risk not captured by existing algorithms and help guide precision medicine approaches.

The strengths of this study lie in the application of PCA to gain insight into both novel and traditional biomarkers of CVD risk in patients with T2DM. This comprehensive statistical approach enables insight into different metabolic pathways. Another strength of the study is the measurement of 24-hour UAE, which is regarded as the gold standard for assessing albuminuria because it accounts for short-term within-person variability (35).

Despite these strengths, this study has several limitations. Given the study's cross-sectional design, causal relationships between the examined biomarkers and cardiovascular risk cannot be established. The single-centre design may limit the generalizability of the findings. The relatively small sample size and the lack of data on medication use might have limited the detection of weaker associations.

Future studies should prospectively validate the role of RBP4 and UAE as adjunctive markers in SCORE2-Diabetes risk assessment and confirm these findings in multicentre prospective cohorts. Longitudinal studies are also warranted to determine whether reductions in serum RBP4 levels or albuminuria translate into improved cardiovascular outcomes. Moreover, integrating multiple biomarkers reflecting inflammation, metabolism, and renal injury with machine learning algorithms might further refine cardiovascular risk prediction. Finally, interventional studies evaluating therapies that directly target RBP4 pathways or albuminuria beyond standard care are of great importance.

Conclusion

Our findings underscore the importance of RBP4 and UAE in predicting cardiovascular risk in individuals with T2DM. Incorporating these biomarkers into existing risk models such as SCORE2-Diabetes might enhance early cardiovascular risk detection and guide personalized preventive strategies. Future research should validate these results in larger populations and evaluate interventions targeting these pathways.

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Authors' contribution

All the authors contributed to the study conception and design. AK and BB performed material preparation, data collection, and laboratory analyses. Statistical analysis was performed by NM and PK. AK and FM wrote the first draft of the manuscript. All the authors have read and approved the final version of the manuscript.

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Ethics Approval

The study protocol was approved by the Institutional Ethical Committee (number 317/2-3). The investigation was conducted in accordance with the Declaration of Helsinki.

Consent to participate

Each participant provided informed consent.

Consent for publication

Written consent for publication was obtained from all participants. Each participant gave explicit permission for the use of their anonymized data in this study and its dissemination in scientific publications.

Data availability statement

All data generated or analysed during this study are included in this article. The data will be available upon reasonable request (contact person: aleksandranklisic@gmail.com).

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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